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A Critical Review.

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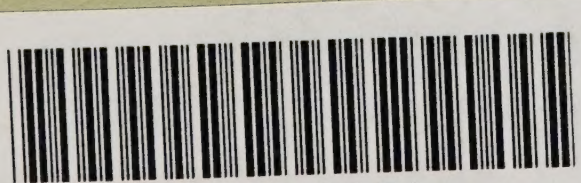
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RECENT WORK ON THE PATHOLOGY OF BLACKWATER FEVER: A CRITICAL REVIEW.

By WARRINGTON YORKE, M.D.,

Sectional Editor, Tropical Diseases Bureau.

Although during recent years a considerable number of papers dealing with blackwater fever have been published, very few of them are of any scientific value, and our state of ignorance regarding this important disease is almost as great as it was ten to fifteen years ago.

Broadly speaking, recent communications on blackwater fever can be grouped into the four following classes :—

1. Papers of an essentially clinical nature, in which are recorded, sometimes in remarkable detail, the various symptoms and signs observed by the author. Such papers, which have appeared to the reviewer as merely unnecessary, are, in the majority of instances, the efforts of those who have but recently visited the tropics—usually as the result of the War—and whose interest has been aroused by this most impressive disease. Knowing little or nothing of the condition and having no access to literature beyond one or two textbooks, they have not hesitated to commit their observations and impressions to print, failing perhaps to realize they are simply repeating what has been written over and over again during the past twenty years.

2. Papers mainly concerned with the endemicity of the disease. In these an attempt is made to show by statistics the relation of the disease to malaria or to the various kinds of malaria infection, to quinine, or to the length of sojourn in the tropics, to map out its race incidence, and so forth. These papers almost invariably fail to be of value because the information they contain is deficient in one or more essentials. All work on endemicity must be based on a census of the population, and this is practically never forthcoming. How often do we read of statements to the effect that the liability to an attack of blackwater fever is greater in persons infected with malignant tertian parasites than in those infected with simple tertian or quartan parasites, or that blackwater fever is more likely to occur amongst those who have been a certain length of time in the tropics than amongst those who have been a greater or less time. These statements are based on the particular parasites present in so many cases of blackwater

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fever, or on the fact that the greatest number of cases of blackwater fever were found amongst those who had lived in the tropics for a certain length of time. As STEPHENS (1922) has recently pointed out, no conclusions regarding liability to attack can be drawn from data of this sort unless we have the population data as well. That is, before we can state that the liability to an attack of blackwater fever is greater in persons infected with malignant tertian parasites than in those infected with the other malaria parasites, we must know not only the particular parasites present in the blackwater cases, but also the number of persons infected with each of the three varieties of parasites in the region under consideration. For example, if malignant tertian malaria is nine times as frequent as quartan in a certain district, then the observation that malignant tertian parasites were found in 90 per cent. of blackwater cases and quartan parasites in only 10 per cent. obviously does not warrant the conclusion that malignant tertian malaria renders a person more liable to an attack of blackwater fever than does quartan malaria ; but if, on the other hand, the malaria census of a district shows that malignant tertian and quartan are approximately equally common, then an observation that malignant tertian parasites were found in 90 per cent. of blackwater cases and quartan parasites in only 10 per cent. would warrant the conclusion that malignant tertian malaria renders a person more liable to an attack of blackwater fever than does quartan malaria. Similarly, an observation to the effect that 60 per cent. of the cases of blackwater fever in a certain colony occurred amongst persons who had been two years in the colony and only 10 per cent. among those who had been five years in the colony does not warrant the conclusion that one is more liable to malaria during his second year of sojourn in the colony than during his fifth year, unless we have information regarding the length of sojourn of the susceptible population in the colony. If such a census should show that six times as many persons were in their second year of sojourn in the colony as there were in their fifth year, then the liability to blackwater is really the same in the second and fifth years.

3. Papers dealing mainly with the treatment of the disease. Such papers are in the main without any value and the claims made quite unjustifiable. Time and again papers have appeared in which a more or less complicated line of treatment is described in detail, and then it is naïvely recorded that several cases were treated successfully by it. The authors are apparently blissfully ignorant of the fact that about 75 to 80 per cent. of cases recover whatever treatment be adopted, and that in view of this the fact that three or four cases have survived any particular drug or combination of drugs is merely a *post hoc* phenomenon and does not justify the belief that they have survived because of the drugs administered.

4. Papers dealing with the pathology of the disease. This group comprises a relatively small proportion of the total papers, and it is with it that I propose to deal mainly in the present review.

Nearly two years ago A. PLEHN (1920) published a most interesting paper on the genesis of blackwater fever. In the introductory portion of his paper PLEHN states briefly that there is in German scientific circles no longer any doubt but that blackwater fever is merely a complication of malaria : the presence of malaria parasites in the body is essential for the development of blackwater fever. Owing to repeated destruction of parasites during the febrile attacks, the body

becomes super-sensitive to the malaria parasite protein. When this super-sensitiveness has attained to a sufficiently high degree, a further solution of quite a small number of parasites may precipitate the crisis ; but, as a rule, however, this only occurs when numerous parasites are suddenly destroyed. In most cases the decisive cause of the destruction of the parasites is quinine, but sometimes it is some other drug given as a substitute for quinine or merely with a view to combating symptoms, *e.g.*, tuberculin, antipyrin, phenacetin, phenokoll, methylene blue, salvarsan, atophan, and the application of X-rays to the spleen ; in quite rare cases there is no recognizable cause, even the constitutional disturbances due to cold, wet, wounds, or over-exertion, etc., being absent. The sole essential for the production of blackwater fever is an acute dissolution of a certain number of malaria parasites. The condition of super-sensitiveness appears to be transitory and it becomes compensated through antibody formation by the organism.

PLEHN does not in this paper discuss the evidence supporting the view that blackwater fever is of malarial origin, and the subject is outside the scope of this critique, but attention might here be drawn to an admirable article by STEPHENS (1913), in which the question is fully considered.

The main portion of PLEHN's paper deals with the mechanism by which two of the most important symptoms of the disease are produced, viz., haemoglobinuria and jaundice. In conducting this enquiry PLEHN attempts to answer three questions :—

1. Where does the haemolysis take place ?
2. What is the origin of the casts in the renal tubules ?
3. How is the jaundice produced ?

With regard to the first of these questions, PLEHN reaches the conclusion that there is no evidence that haemolysis takes place in the blood-stream, because, firstly, haemoglobinaemia is not, as a rule present in blackwater fever, PLEHN himself failing to find haemoglobin in the serum even at the time of maximum haemoglobinuria ; secondly, haemolysin is not found in the blood of blackwater fever patients ; and, thirdly, there are no indications of changes in the red blood cells, *e.g.*, shadows, etc., which might be expected if 60–80 per cent. of the red cells were destroyed in the blood-stream within 24 hours.

PLEHN further finds no support for the hypothesis that haemolysis takes place in the organs which are commonly supposed to be concerned with the physiological destruction of erythrocytes, *e.g.*, spleen, liver and bone marrow, the capacity of which in this direction might be supposed to be increased in blackwater fever, especially if directed against erythrocytes of lowered resistance. The blood destruction is too great and too quick to be explained by the chronic pathological changes seen in these organs in old malaria patients. Again, the weight of evidence does not support the view that the blood-forming organs are exhausted as the result of being continually called upon to replace the destruction due to malaria, as experience has shown that chronic blood destruction by poisons has the contrary effect—increasing and not decreasing new cell formation. The resistance of the erythrocytes in blackwater fever to anisotonic NaCl solutions is lessened in only a proportion of cases—sometimes it is increased ; and, furthermore, such changes are specific only to NaCl solutions, and do not necessarily hold for blood poisons such as saponin. Finally, if haemolysis did take place in the organs in question, then the haemoglobin in order to reach the kidneys must pass via the circulation, and, as already remarked, this does not occur.

All these considerations, states PLEHN, allow of scarcely any other possibility than that the haemolysis takes place in the kidneys. After remarking that nothing is known concerning a haemolytic function of the healthy or diseased kidneys, and that, as a general rule, there is no sign of inflammation in the kidneys of blackwater patients, PLEHN passes on to consider the second question, viz., what is the origin of the cylindrical plugs in the renal tubules ? He points out that these consist either of agglomerations of round, structureless, more or less strongly acid-staining (eosin, picric acid) granules, which in the same conglomerate are of about equal size, but which vary in different plugs between the size of a red cell and that of fine detritus ; or they consist of compact, almost homogeneous, cylinders which in parts show fine granulation, the affinity of which for acid dyes is mostly slight. PLEHN considers that these plugs do not arise from the red blood cells, because these are almost invariably absent from the urine of blackwater patients, and because they take up stains to such a varying degree. The most generally accepted view is that they arise from coagulation of a highly albuminous, haemoglobin containing, exudate ; but on this hypothesis it is difficult to explain why some of the plugs are uniformly more or less granular, whilst others, and apparently the oldest, are almost homogeneous. Weigert's fibrin and elastic stain does not show the fine network characteristic of coagulation. In order to solve this question, which, according to PLEHN, is more than twenty years old, PLEHN re-examined his collection of pathological material. He found that certain lines, which appeared to be the walls of capillaries, and in which endothelial nuclei could be clearly distinguished, bordered not only blood-vessels but also renal tubules which had lost their epithelium. Whether the basement membrane was disintegrated or whether it was so closely applied to the capillary wall as to be indistinguishable from it was undecided. The fragments of the epithelial cells are obviously the material from which the plugs are formed, as one can distinguish in the same kidney all stages from more or less loose epithelial masses, some of which still exhibit nuclei and some of which do not, to large granular and very fine granular, almost homogeneous, firm cylinders. This affinity for acid stains is due to saturation with blood-colouring matter ; in the oldest plugs it is usually slight, but sometimes it is as strong as that of the blood-cells themselves. PLEHN points out that these epithelial fragments are often not found in the place where they have undergone degeneration, but further down the tubules in a region where the epithelial wall is complete. It is noteworthy that these plugs are only occasionally seen in the urine, probably because the urine-stream in the affected tubules very soon is held up. The brown finely granular detritus found in the urine is likewise derived from disintegration of epithelial cells and not from the red cells.

If the renal tubules which contain these cylinders are followed backwards towards the glomerulus a point is often reached where a renal tubule appears to communicate directly with a capillary, *i.e.*, at a point where the epithelium has completely disappeared. It is noteworthy that the number of tubules which have lost their epithelium appears small in comparison with the quantity of plugs, but this is due to the fact that the former are easily overlooked.

As indicative of the functional disturbance of the kidneys in blackwater fever, the inability to excrete bilirubin is of the first importance ; even in the most severe icterus bilirubin is not found in the urine, although it can readily be demonstrated in the blood serum. Moreover,

notwithstanding the high albumen content even in conditions of oliguria, the specific gravity of the urine is extraordinarily low—urine of 1,005 and 1,004 is quite usual, and even 1,002 and 1,001 occur. The specific gravity of urine depends mainly in the first place upon its NaCl content, and in the second place upon its urea content. Finally, the functional insufficiency extends under certain circumstances to the excretion of water ; oliguria is common, and complete anuria may occur with retention of all the waste products of metabolism.

From these considerations PLEHN concludes that : (1) owing to the loss of epithelium at various places along the renal tubules, and possibly also of the basal membrane, the relationship between the contents of the blood-vessels and renal tubules becomes abnormal, and under certain circumstances a direct admixture takes place ; and (2) owing to its high degree of hypotonicity the renal secretion in blackwater fever has a haemolytic action, which normal urine has not.

With regard to the third question, viz., the method of production of the jaundice, PLEHN states that up to the present we have adhered to the hypothesis of its purely hepatic origin. Icterus cannot be due to obstruction in the large bile ducts, because the intestinal contents are usually rich in bile. Neither can it be explained on the assumption that a surfeit of free haemoglobin reaches the liver through the general circulation or through the portal system from the spleen, because, as already stated, haemoglobinaemia is usually absent from the general circulation and consequently cannot be produced in the spleen, because the path from the spleen to the kidneys, where it is excreted, is through the general circulation. The kidneys, moreover, where the solution of haemoglobin occurs, drain into the vena cava.

Recently, however, the exclusively hepatic genesis of the icterus has been questioned, and with good reason, since the so-called reticulo-endothelial cells—especially those of the spleen, but also those of the bone-marrow, lymph-glands and elsewhere, possibly even the blood-stream itself—are prominently concerned with the formation of bilirubin (LEPEHNE, 1919).

Probably the haemoglobin which is not directly passed out into the urine escapes back from the kidneys into the blood-stream and is taken up by these cells before it accumulates sufficiently to be recognizable. The reticulo-endothelial cells convert it into bile pigment and throw it back into the circulation, where the bilirubin accumulates, because the kidneys are unable to excrete it, and consequently it is deposited in the tissues, giving rise to icterus.

Perhaps, however, the mechanism is quite different, as we must bear in mind the fact that in many cases of malaria in the Cameroons bile is vomited by the litre without any considerable decrease in the haemoglobin content of the blood.

Sometimes in blackwater fever icterus is only very slight, and this is the rule in the worst cases, where the blood destruction is so great as rapidly to result in death without the development of icterus, and also in very slight cases, where the haemoglobinuria is only observed fortuitously and is not accompanied by other symptoms. The absence of jaundice in both these classes of cases can be explained on the assumption that the haemoglobin is completely excreted by the kidneys, none getting back into the circulation.

Summarizing PLEHN's theory of the pathology of blackwater fever, it appears to be briefly as follows :—

The primary lesion, resulting from a sort of anaphylactic shock in a malaria subject, is profound changes in the renal epithelium, which

here and there becomes dislodged, and disintegrates, sometimes taking the basement membrane with it. These dislodged, degenerate cells (impregnated with haemoglobin) collect in the lumen of the tubules and form the casts.

These histological changes in the kidneys are accompanied by equally profound loss of function, the power of eliminating urea and sodium chloride, and even water, being greatly decreased. The inability of the kidney to excrete bilirubin is especially noteworthy.

As a result of the dislodgment and degeneration of epithelium and basement membrane, communication occurs between the capillaries and tubules, and owing to the absence of urea and salt the urine is hypotonic and lakes the blood. The jaundice is explained on the assumption that some of the haemoglobin escapes back from the renal tubules into the blood-stream, where it is at once taken up by certain reticulo-endothelial cells and converted into bilirubin, which is disgorged into the circulation, and, as the kidneys are unable to secrete it, deposited in the tissues.

This theory is not only ingenious and attractive, but sufficiently plausible to merit serious attention. In subjecting it to critical examination, we must obviously consider the premises on which it is based. It might at once be stated that PLEHN seems to have relied chiefly upon his own observations and on those of certain German workers, and has consistently ignored the work of foreign observers—particularly English and American—to which, with a single exception, no reference is made. Had PLEHN taken the trouble to familiarize himself to a greater extent with the work of others, it is probable that he would have avoided certain of the errors into which he has apparently fallen.

The fundamental premise on which the whole of PLEHN's theory rests is that, as a rule, haemoglobinaemia does not occur in blackwater fever. There is, unfortunately, astonishingly little in the way of precise information on this important question. So long ago, however, as 1901, STEPHENS and CHRISTOPHERS record that they observed haemoglobinaemia in two of their Sierra Leone cases and in another case it was doubtful ; whether they examined for it in the remaining five cases is not stated, and no reference is made to the subject in the protocols of the cases. CHRISTOPHERS and BENTLEY (1908) write as follows :—

" Examination of the blood in blackwater fever has shown without exception the presence of true haemoglobinaemia, demonstrated by the centrifuging of blood received into hypertonic citrate solution ; the serum after clotting has also always shown haemoglobin, provided the haemoglobinuria was still in progress, but in both cases the amount was usually small and more or less masked by the extraordinarily intense yellow coloration of the serum. We have only once seen haemoglobin so great in amount as to give a rosy colour to the serum and its presence is generally only indicated by an orange tint. Owing to this fact it is difficult to estimate with accuracy the amount of haemoglobin present ; but in most cases seen by us it does not seem to have been more than the equivalent of a 1 per cent. solution of normal blood. In the one case in which the serum was rosy red the percentage of haemoglobin was as high as a 3·75 per cent. solution of blood."

BARRATT and YORKE (1909) investigated this subject with extreme care, and made precise estimations of the amount of dissolved haemoglobin in the plasma of seven healthy persons, 21 suffering from malaria and other diseases, and in 17 cases of blackwater at various stages of the disease. In the healthy persons the amount of

haemoglobin dissolved in the plasma varied between 0·1 per cent. and 0·15 per cent.* Among the 21 persons suffering from malaria, dysentery, etc., the amount of dissolved haemoglobin in the plasma ranged between 0·02 per cent. and 0·2 per cent., except in two cases of malaria, where the somewhat higher degree, 0·31 per cent., was observed. Among the 17 cases of blackwater 40 estimations were made. The observations fell into three classes : those made before, after, and during the passage of blackwater. In the first class, which includes only three observations, the percentage of dissolved haemoglobin was 0·3 or less ; in the second class, consisting of 19 observations, the percentage of dissolved haemoglobin was in 12 cases raised, lying between 0·3 and 0·95 . ; in the remaining seven observations it lay between the limits met with in health, viz., 0·09 per cent. to 0·25 per cent. With regard to the latter, it must be remarked that although in these observations the first specimen of urine voided after the examination of the blood plasma contained haemoglobin, it does not necessarily follow that the urine which was being excreted at the time of the examination of the blood plasma contained haemoglobin. It is possible, if not probable, that in some cases the haemoglobinuria had already ceased, although, owing to the relatively long intervals at which the patient passed urine, this did not become evident. Barratt and Yorke sum up by stating that " In the observations made in blackwater fever, while the urine in the bladder still contained haemoglobin, an increased amount of dissolved haemoglobin was observed in most but not in all cases."

Arkwright and Lepper (1918) record that in four of the ten cases of blackwater fever examined by them the serum was sufficiently coloured with haemoglobin to give a spectrum in a tube 0·3–0·4 cm. in diameter ; citrated plasma was examined in two of these cases and was also found to contain haemoglobin. The remaining six cases were not altogether satisfactory, because in three the serum was examined only a few hours before the haemoglobin ceased, in one during an intermission, in one after the haemoglobin had ceased, whilst the last, which was examined only a few hours after the haemoglobinuria had commenced, was a mild case. Further information is given regarding one of the four positive cases, Arkwright and Lepper stating that in this case both the serum and the plasma were examined on the first and second day of the attack and both were a deep brownish crimson on each occasion. The colour was due to methaemoglobin, probably with some oxyhaemoglobin, and also, in part, to bile pigment.

Dudgeon (1920) found that the blood withdrawn from a vein in a case of blackwater fever may show evidence of haemolysis varying from a deep coloration of the plasma to a faint tinging. He adds that " the evidence of haemoglobinaemia rapidly disappears from the blood, so that this phenomenon may be overlooked unless the examination is made at the correct moment."

There is, hence, considerable evidence to show that haemoglobinaemia in amounts definitely exceeding that found in the blood of normal

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* Barratt and Yorke used wet human red cells as their standard, and when they state that serum, etc., contains a certain percentage of haemoglobin, they mean that there is in it as much haemoglobin as in a solution containing a similar percentage of normal human erythrocytes. Hence, in order to compare Barratt and Yorke's figures with those of other observers who used normal human blood as their standard, it is necessary to multiply Barratt and Yorke's figures by the fraction $\frac{5}{2}$, the erythrocytes constituting about 40 per cent. by volume of normal blood.

persons, or of persons suffering from malaria, dysentery, etc., is present in a considerable number of cases of blackwater fever, and it is highly probable that, as BARRATT and YORKE, and DUDGEON, pointed out, the failure to recognize haemoglobinaemia may in many, if not all, cases be due to the fact that the attack was really over (*i.e.*, passage of haemoglobinuria had ceased) at the time the observation was made, even though the next specimen of urine voided contained haemoglobin. It is obvious that we require more precise information on this important question, and not merely vague statements that haemoglobinaemia was present or absent, but exact quantitative estimations, such as those made by BARRATT and YORKE, which should, so far as is possible, be correlated with records regarding the degree of haemoglobinuria excreted at the time the observation was made. For this purpose the use of a catheter will probably be necessary.

Although information on the point is, up to the present, not so complete as can be desired, there appears to be no justification for PLEHN'S assertion that haemoglobinaemia is, as a rule, not present in blackwater fever.

PLEHN'S statement that haemolysis occurs in the renal tubules as a result of admixture of blood from the capillaries with hypotonic urine requires very careful examination.

Leaving aside for the moment the precise nature of the renal lesions and their origin, and, admitting for the sake of argument that a certain amount of haemorrhage into the uriniferous tubules does take place, we must enquire whether the available facts justify PLEHN'S contention that it is here that the erythrocytes are haemolysed, owing to their contact with hypotonic urine. PLEHN apparently bases his hypothesis mainly on a belief that the specific gravity of the urine in blackwater fever is extraordinarily low (1,005–1,004 or even less), chiefly owing to its deficiency in NaCl and urea ; although he also recognizes as a possible secondary cause the existence of haemolytic substances of the nature referred to by MATKO (1918).

BARRATT and YORKE (1909), who investigated the action of urine on erythrocytes very fully, write as follows :—

" If an emulsion of red blood cells in 0·9 per cent. solution of sodium chloride is added to urine, it is seen that the latter, when the changes in the red cells described below become advanced, gradually turns brown in colour, but no reddish tint is noticeable. Urine does not produce visible laking of red blood cells unless its specific gravity is less than 1,009. Urine of specific gravity 1,002 lakes red blood cells as rapidly as distilled water. Red blood cells in urine whose specific gravity exceeds 1,009 become, on standing, poorer in haemoglobin and assume a darker, somewhat brownish, aspect, but are ultimately decolorized and form a brownish-white precipitate at the bottom of the tube in which the urine is contained. If such urine at any time after adding the red blood cells is centrifugalized and the supernatant fluid examined spectroscopically, it is found that no oxyhaemoglobin bands are at first seen, the red blood cells becoming decolorized without any laking of their haemoglobin. When, however, the red blood cells have lost 75 per cent. or more of their haemoglobin, oxyhaemoglobin bands make their appearance in the urine, which is now brownish in colour. Just as occurs when dissolved haemoglobin is added to urine, a precipitate brown in colour may make its appearance at the end of 24 hours in the mixture of urine and red cells. It is thus seen that in the red cell the haemoglobin is broken up by urine of specific gravity exceeding 1,009 *in situ*, and does not leave the cell until it has become completely converted into brown pigment."

The question now arises : Is PLEHN correct when he states that the urine in blackwater fever is of extraordinary low specific gravity ?

On looking up the point in the various textbooks one finds nothing beyond the general statement that the specific gravity of the urine is rather low, *vide* ZIEMANN in MENSE's Handbuch, CASTELLANI and CHALMERS, etc. On searching through the literature, a few more or less isolated records are found. KRAUS (1904) gives details of a case in which determinations of the specific gravity were made in eight specimens of urine passed during the first two days of the illness ; the figures varied between 1,034 and 1,016 ; determinations of five other specimens made during the third and fourth days gave results varying between 1,012 and 1,006.

CHRISTOPHERS and BENTLEY (1908) examined the specific gravity of the urine passed by one of their cases during the first three days of the illness and found that it varied between 1,017 and 1,020. CHRISTOPHERS and BENTLEY then add that if the urine were hypotonic, any haemorrhage into the urinary passages could give rise to a condition resembling haemoglobinuria—a theory apparently advanced by PELLERIN as long ago as 1865 and 1876. With a view to examining the question, CHRISTOPHERS and BENTLEY estimated the haemosozic value of the urine in a number of their cases and found that even when haemoglobinuria is present it is far above that at which red corpuscles are haemolysed, and consequently that the urine could not on account of its osmotic effect dissolve red cells.

BREM (1911) gives information regarding the specific gravity of the urine in one of his cases. Eight determinations were made during and after the attack over a period of four days ; the readings obtained varied from 1,033 to 1,022.

DEADERICK (1910) states that the average of a large number of urine analyses gave a specific gravity of 1,015. PARSONS and FORBES (1919) give information regarding three mild cases of blackwater fever. The specific gravity of the urine in the first case varied from 1,023–1,015, in the second from 1,030–1,010, and in the third from 1,020–1,010.

By far the most complete records on the subject are, however, those of BARRATT and YORKE (1909), who examined the specific gravity of almost every specimen of urine passed by the cases which came under their observation. Critical examination of their records show that there is no justification for the statement that the specific gravity of blackwater urine is extraordinarily low or, in fact, lower than normal. The specific gravity varied considerably in different specimens of urine from the same case ; very few observations of 1,009 or less are recorded, and these low figures are in no way related to the presence in the urine of haemoglobin. This fact is particularly well illustrated by Case 10 of BARRATT and YORKE's series, in which the specific gravity was determined of almost every specimen of urine passed by the patient over a period of 14 days, during which time the patient had, in addition to the initial attack of haemoglobinuria, which lasted three days, 11 distinct relapses, between each of which the urine was of normal colour and appearance. The 85 determinations made on this case show that, although the specific gravity varied between 1,024 and 1,002, only six of the readings were below 1,010, and these low readings did not correspond to the haemoglobinuria attacks, but, on the contrary, were all made during the interval periods.

There appears then no justification for the general statement that the specific gravity of the urine in blackwater fever is abnormally low. It varies considerably, just as does that of the urine of normal persons, depending on a number of factors, one of the most important of which is the quantity of fluid ingested.

Another fact which tells against PLEHN's theory of the haemolytic action of the urine is that it is by no means uncommon to find unlaked erythrocytes in scanty numbers in the urine of blackwater cases. KRAUSS (1904) records that red cells were present generally in small numbers in seven, or possibly nine, of his 17 cases ; PARSONS and FORBES (1919) found them in two of their three cases, and DEADERICK in five of 34 cases. BARRATT and YORKE (1909) record that red blood cells were encountered during the haemoglobinuria of blackwater fever in 10 of the 20 attacks investigated, they were usually found in small numbers only ; in three cases red cells were not found at first, but as haemoglobinuria was passing off they made their appearance. DUDGEON (1920) writes that red cells which are present in relatively small numbers rapidly disappear from the urine in blackwater fever as the acute stages of the disease subside, but by accurate examinations of centrifuged samples of urine, red cells are found to occur more frequently in the acute stages of the disease than is generally believed and taught. ARKWRIGHT and LEPPER (1918) state that red blood corpuscles were usually absent from the urine, but a few were found in two of their cases.

Finally, as militating against PLEHN's theory that haemolysis takes place in the renal tubules, reference should be made to the fact that the amount of haemoglobin passed out in the urine in blackwater fever is very small compared with the destruction of blood which takes place. BARRATT and YORKE (1909) determined in 10 of their cases the total amount of haemoglobin passed into the urine and found that it varied between 1·5 gm. and 75 gm. of wet red cells ; when these amounts are considered with the haemocrit readings of the blood of the various cases, it will be seen that they represent a comparatively small fraction of the total loss of blood. ARKWRIGHT and LEPPER (1918) found that in one of their cases the total haemoglobin lost in the urine was equivalent to 11 oz. of normal blood, and remark that a loss of this order does not seem nearly sufficient to account for the high degree of anaemia produced by the disease.

This fact, while negativing PLEHN's theory, is quite in harmony with the theory that the haemoglobinuria is dependent on haemoglobinaemia, as it has been shown experimentally by YORKE and NAUSS (1911) that only one-fifth or less of the haemoglobin injected intravenously in rabbits is excreted by the kidneys. The following year a similar result was obtained by PEARCE, AUSTEN, and EISENBERG (1912), who found that after rapid injection into dogs of more than 0·012 gm. per kilo per minute of haemoglobin, 16 to 36 per cent. of the total amount, if this equals 0·25 gm. per kilo, is eliminated in the urine ; whereas if the injection of not more than 0·35 gm. per kilo is made slowly (less than 0·01 gm. per kilo per minute), the amount eliminated in the urine is only 2·33 to 9·5 per cent. of the total injected.

The facts available therefore, do not support PLEHN's theory that the haemoglobinuria results from the laking of blood which escapes into the renal tubules ; the specific gravity of blackwater urine is not, as PLEHN asserts, abnormally low during an attack, unlaked red cells are frequently found in small numbers in blackwater urine, and, finally, the amount of haemoglobin found in the urine represents only a small fraction of the total haemolysis which occurs, as judged by the lowering of the haemocrit reading or red cell count.

Let us now consider PLEHN's hypothesis that, as a primary result of the blackwater toxaemia, or shock, the epithelial cells lining the

renal tubules are profoundly affected, that they degenerate and become dislodged, and finally disintegrate, giving rise to the material from which the plugs are formed; and, furthermore, that the functional disturbances of the kidneys (oliguria, anuria, etc.) are the direct result of these changes in the epithelium.

This view is directly opposed to that expressed by WERNER (1907) and by BARRATT and YORKE (1909), and by many more recent authors, who found that the granular plugs are derived from haemoglobin, that there is no marked degeneration and disintegration of the epithelium, that dislodgment of the epithelium is a secondary matter, and that the functional disturbances are the result of mechanical obstruction of the tubules by the plugs.

In view of PLEHN's statements, the subject has quite recently been reinvestigated by SALVIOLI (1922), who, as the result of microscopic examination of the kidneys of 13 cases of blackwater fever, in the course of which he employed, in addition to the ordinary methods, that devised by MILLER (1911) for elective staining of haemoglobin, finds himself unable to confirm PLEHN's observations. Pronounced degenerative changes in the epithelial cells were observed in only two cases, and in those the excretion of haemoglobin was insignificant. The epithelial cells in the proximal portion of the uriniferous tubules were unchanged in 11 cases, the loss of epithelium was mechanical, due to friction of the plugs, and the dislodged cells were normal. Blood was found only in the blood-vessels and the corpuscles were normal; traces of blood destruction were not found. The most important finding was the filling of the tubules with granular, or flocculent, masses and widening of the lumen, and here and there in the distal portion of the tubules, dislodgment of the epithelial cells and thick coagulation masses. The majority of the preparations showed that the masses found in the distal part of the tubules originated either out of haemoglobin-impregnated substances or out of coagulation masses mixed with drops of pure haemoglobin.

The experimental work recorded by the reviewer in 1911(*a*) affords strong support to the view that the epithelial changes in the kidneys of blackwater fever patients are secondary to the passage through them of haemoglobin and not, as PLEHN considers, precursors of the haemoglobinuria. By introducing into the circulation isotonic solutions of haemoglobin prepared from the animals' own erythrocytes, the reviewer succeeded in producing in rabbits renal changes identical with those found by WERNER, and by BARRATT and YORKE, in the kidneys of blackwater fever patients, and, furthermore, suppression of urine and death from uraemia. He summarized this observation as follows:—

"The immediate result of such injections is a diuresis, dependent upon the action of the sodium chloride solution used for dissolving the haemoglobin. At the same time the renal epithelium commences to excrete haemoglobin into the tubules. At first this is quickly carried away by the rapid secretion of watery urine from the glomeruli. The result is a considerable flow of clear urine containing haemoglobin in solution. As the diuresis subsides, the haemoglobin, or a closely related derivative, becomes deposited to a certain extent in the tubules. At this stage the urine is less in amount and contains a quantity of solid material, consisting chiefly of granular casts and débris. Still later, especially when the degree of haemoglobinaemia is high, epithelial cells are shed from the lining membrane of the renal tubules. This is probably dependent upon one or both of the following factors. In the first place it is possible that the excretion of large quantities of a foreign substance like haemoglobin may

be attended by injurious effects upon the renal epithelium. In the second place the presence of casts in the lumen of the tubules would tend to damage the lining epithelium mechanically, either by friction as the plug was forced down the tubules owing to the *vis a tergo* of the glomerular secretion, or by causing a complete or partial block of the tubule, and, as a result, dilatation of that portion above the plug, and, consequently, separation of the epithelium from the basement membrane.

"This stage represents the commencement of the development of suppression of urine. Gradually, as haemoglobin continues to be excreted by the renal epithelium more and more tubules become occluded. The situation in which complete blocking of the lumen first occurs appears to be, as a rule, the narrow tubules of Henle. Coincidently, with the plugging of the tubules there is a marked diminution in the amount of urine voided, and also of the haemoglobin it contains. The condition steadily progresses, until, finally, complete anuria results. After cessation of the flow of urine has lasted for a certain time, a gradual re-establishment of urinary secretion occurs. The urine is loaded with albumen, and is exceedingly turbid. On centrifugalization a large deposit, sometimes equalling as much as one-fourth of the volume of urine, is thrown down. This deposit consists of granular casts, epithelial casts, granular débris and degenerated epithelial cells. Urine of this nature continues to be passed until the animal's death. During the period of total or partial anuria marked changes occur in the kidney. Owing to the secretion of the glomeruli those portions of the tubules above the plugs become enormously distended with fluid. As a result, the epithelium lining these portions becomes extremely thin, and in places entirely disappears. The dilated tubules press upon adjacent tubules, so that on microscopic examination the latter appear of small size, and their lumina are completely obliterated.

"After a time, owing to gradual disintegration of the casts plugging the tubes, the urine commences to escape into the ureter once more.

"The serum proteins and red blood cells pass through the damaged epithelium and those portions of the tubules devoid of epithelial cells, with the result that the urine is loaded with albumen. Although the casts may be to a great extent washed away in the course of the next few days, yet the renal tubules remain dilated and the serum proteins continue to escape into the urine until the animal's death, which in these cases is probably due to uraemia."

In a later paper (1911(*b*)) the reviewer showed that haemoglobin is secreted by the epithelial cells lining the tubules and not by Bowman's capsules.

If the red-water of dogs infected with *P. canis* be examined, it will be found to contain in considerable quantity the finely granular casts and detritus so characteristic of blackwater fever urine. A similar observation has been made repeatedly by the reviewer in the urine of patients suffering from paroxysmal haemoglobinuria. Now, in both these conditions haemoglobinaemia is a well-marked phenomenon, and there is no reason to doubt that it precedes and is the cause of the haemoglobinuria. As a result of examination of the kidneys of dogs infected with *P. canis*, the reviewer (1911) concluded that granular material was derived from the haemoglobin excreted through the epithelium of the renal tubules.

From this discussion it will be seen that the bulk of pathological and experimental evidence is overwhelmingly against PLEHN's theory of a primary renal lesion with haemoglobinuria as a secondary phenomenon. Profound degeneration of the renal epithelial cells is not found and the granular detritus and casts seen in the urine and renal tubules are not derived from disintegrated epithelial cells. Dislodgment of the epithelial cells, and such degeneration as does occur is a

secondary matter and probably due, in part at least, to mechanical causes. The serum proteins and red cells which are found in the urine in severe cases of blackwater gain access to the tubules through the lesions produced by dislodgment of the epithelium.

In conclusion, it must be pointed out that although it is clear that a sudden, and often enormous, haemolysis occurs in blackwater fever, nothing is known regarding the mechanism by which it is produced or where it takes place. In paroxysmal haemoglobinuria the work of DONATH and LANDSTEINER (1904), WIDAL and ROSTAINE (1905), EASON (1906) and others has shown that haemolysis takes place in the peripheral blood and that cold is the exciting cause. The precise mechanism of autolysis in this condition has recently been studied in great detail by YORKE and MACFIE (1921), and a simple method of determining the existence of the autolysin described. No autolysin has, however, been found in the blood of blackwater fever patients (BARRATT and YORKE, and others). DUDGEON (1920) records that he has succeeded in demonstrating a haemolytic action of acetone and alcohol extracts of the spleen, kidneys and urine of patients who have died of blackwater, and that this substance was not found in the urine and organs of persons who had died of other diseases; and NIERENSTEIN (1919) states that he has found in the urine of blackwater patients a derivative of quinine—haemoquinic acid—which exerts a powerful haemolytic action. These observations, however, require confirmation, and in any case their bearing on the haemolysis which occurs in blackwater fever is far from clear.

Very many observations have been made by different authors to determine whether in blackwater fever there is any abnormal sensitiveness of the erythrocytes to hypotonic saline, quinine, etc. The general consensus of opinion is undoubtedly that there is none. CHRISTOPHERS and IYENGAR (1915) conducted a long series of experiments on the effect of haemolytic drugs, toxins and antisera upon the haemolytic point and the association between this point and haemoglobinuria. They found in their experiments, which were conducted upon rabbits, that potassium chlorate, carbolic acid, sodium glycocholate, sodium taurocholate, quinine, diphtheria toxin and pyocyaneous toxin neither induced haemoglobinuria nor had any effect on the haemolytic point; those substances, however, which caused haemoglobinuria (pyrogallic acid, abrin and haemolytic serum) raised the haemolytic point, but none of these ever produced haemoglobinuria until its action had resulted in a raising of the haemolytic point to 0·9. CHRISTOPHERS and IYENGAR conclude from this that if in blackwater fever, as is generally agreed, there is no distinct raising of the haemolytic point, this may be taken as indicating that the mechanism of haemoglobinuria in this disease is fundamentally different to that occurring in the haemoglobinuria induced by the injection of a toxic substance or of an artificial haemolysin.

The mechanism by which jaundice, which is usually so prominent a symptom in blackwater fever, is produced, is as yet unsolved. In this disease there is abundant evidence of a great production of bile, *e.g.*, the frequent vomiting of large quantities of material heavily charged with bile, the presence of bile pigment in the serum, and the post-mortem findings of a gall bladder loaded with dark green, sometimes almost black, inspissated bile. It is important to note that although bile-pigment is usually found in the serum it has only occasionally been discovered in the urine. BARRATT and YORKE

(1914) showed experimentally that the introduction of isotonic haemoglobin solutions into the circulation of rabbits resulted not only in a considerable increase in the volume of bile poured into the gall bladder, but also in the concentration of bile-pigment. No jaundice, however, was obtained by this means, nor has any been noted by the reviewer in cases of paroxysmal haemoglobinuria. It appears, therefore, that the mere existence of haemoglobinaemia, even in a degree far exceeding that which has hitherto been recorded in blackwater fever, is of itself insufficient to produce icterus, and that consequently in blackwater fever there must operate some additional factor about which at present nothing is known. Reference should, however, here be made to the very interesting series of papers by PEARCE, AUSTEN, KRUMBHAAR, and others (1912), who showed that under certain conditions jaundice resulted from the intravenous injections into dogs of haemolytic serum and even after injections of haemoglobin.

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